Effect of calcium-free seawater on sea urchin (*Lytenchinus variegates*) gastrulation

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Objective:

To test the effects of calcium-free seawater on gastrulation in sea urchin (*Lytechinus variegates*) embryos during two stages of development.

Background:

Sea urchins are a common indicator organism used in research, quickly showing signs of stress due to changes within their environments ("Sea Urchin Embryology"). We will use this property to assess the affect of removing calcium from their environment during two stages of development.

Calcium plays a significant role in sea urchin development. It is necessary in two processes following fertilization. In the acrosomal reaction, it is involved in the fusion of the acrosomal vesicle and the sperm plasma membrane. This fusion results in the extension of the acrosomal process. The acrosomal reaction is initiated by a fructose-containing polysaccharide within the egg jelly, which binds to the sperm and allows calcium to enter the sperm head. The second mechanism involving calcium is the cortical granule reaction. This reaction involves an increase in the calcium ion concentration within the egg. This causes the cortical granule membranes to fuse with the egg plasma membrane, releasing their contents. Removing the calcium during fertilization would not alter these two processes, as the calcium for these early changes originate from within the egg (Gilbert, 2010).

Later in development, the presence of calcium during embryogenesis allows cadherins to function properly, promoting cell-to-cell adhesion. Cadherins are calcium-dependent adhesion molecules. The embryo contains multiple cadherins, each having adhesive specificity toward its own kind. Cells will sort themselves out when mixed, based on cadherin type and ending in the most thermodynamically stable structure (Gilbert, 2010).

In *Lytechinus variegates*, cadherins have a significant role in epithelial-mesenchymal transitions involving the delamination of epithelial cells. Changes in cadherin localization have been shown to accompany ingression of primary mesenchyme cells. Within the embryo the gut tissue must undergo convergence and extension to create the archenteron. The cells of the archenteron remain attached to each other by adherens junctions containing cadherin. This attachment, mediated by cadherins, may be necessary to maintain the archenteron through convergence and extension (Miller & McClay, 1997).

Procedure:

1. Collect gametes from healthy adult *Lytechinus variegates* and fertilize following Gamete Collection and Fertilization Protocol.
    a. Gamete Collection
        i. Inject 3 mL of 0.5 M KCl to induce spawning.

ii. Using Artificial Sea Water (ASW), collect eggs in a beaker, wash and store at room temperature.
iii. Store sperm at 4 °C in a small test tube after collection in a dry Petri dish.

b. Fertilization Protocol
i. Allow 5 mL of eggs to settle in a test tube after transfer from the beaker. Pipette out all but 2 mL of ASW.
ii. Dilute three drops of sperm in 5 mL of ASW (diluted sperm) five minutes before use. Sperm viability in water is short-term.
iii. Observe diluted sperm with a compound microscope to ensure motility.
iv. Fertilize eggs with 2 drops of diluted sperm.
v. Add ASW until the tube is 90% full, 5 minutes post-fertilization.
vi. Use a depression slide to view 2-3 drops of eggs, 10 minutes post-fertilization.
vii. Observe fertilization envelopes.

2. Divide fertilized eggs into three Petri dishes, control, Treatment A, and Treatment B.
3. Add ASW to the control treatment, label, and move to a secure location to develop.
4. After the fertilized eggs in Treatment A reach the two cell stage (70-90 minutes post-fertilization), remove excess ASW and re-suspend eggs in calcium-free seawater*, take photograph.
5. Allow the fertilized eggs in Treatment B to develop until they reached the blastula stage (about 12 hours post-fertilization).
6. Remove excess ASW from Treatment B and replace with calcium-free seawater.
7. Allow embryos to continue to develop and take photos throughout a 24 hour period. Compare to the control embryos which developed only in ASW.

**To create calcium-free seawater*

NaCl	25.6g
KCl	0.7g
$MgSO_4 \bullet 7H_2O$	11.9g
$NaHCO_3$	0.5g
dH_2O	-to 1 liter

Literature Cited:

Gilbert S. *Developmental Biology: Ninth Edition*. Sinauer Associates, Inc. Sunderland, 2010 pp 73-75.

Miller J. & D. McClay. Characterization of the Role of Cadherin in Regulating Cell Adhesion during Sea Urchin Development. *Developmental Biology*. 1997, 192: 323-339.

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